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Research Article

Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Determination of Cabotegravir and Rilpivirine in Bulk and Injection Dosage Form

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ABSTRACT

A simple, sensitive, specific, accurate, and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of cabotegravir and rilpivirine in injection dosage form, using a Waters Model No.2695 series compact system fitted with Agilent - C18 column (BDS) (150 X 4.6 mm, 5μm) and a mobile phase composed of 0.01N KH_2PO_4 buffer (pH: 4.8): acetonitrile (70:30v/v). The 260 nm wavelength was chosen. The retention times of cabotegravir and rilpivirine were found to be 2.30 and 3.187 minutes, respectively. Linearity was established for cabotegravir and rilpivirine in the range of 25-150 μg/mL and 37.5-225μg/mL respectively: with correlation coefficient (r^2) of 0.9999 for both the drugs. Cabotegravir and rilpivirine were found to have %RSDs of 0.4 and 0.3 respectively, for system precision. The proposed methods intra- and inter-day precision assessments showed a relative standard deviation (%RSD) below the maximum permitted level of 2.0. Accuracy was carried out in triplicate, and the percentage recovery was 100.25% for cabotegravir and 99.79% for rilpivirine, respectively. The limit of detection and limit of quantification for cabotegravir were found to be 0.24 and 0.74 μg/mL, respectively and 1.10 and 3.34 μg/mL, for rilpivirine respectively. Both medications were put to a range of stress conditions, including thermal, acidic, basic, oxidative, and photolytic stress. The findings demonstrated that, with the exception of heat, UV, and neutral environments, considerable degradation was found in acidic, basic, and oxidative conditions where a good separation of drug peaks was seen in the presence of the degradation products. As a result, this technique can be used to quantitatively to analyze cabotegravir and rilpivirine in bulk drug and injection dosage forms.

Keywords: Cabotegravir; RP HPLC; Rilpivirine; ICH guidelines

INTRODUCTION

Cabotegravir[1,2] is chemically (N-((2,4-Difluorophenyl) methyl)-6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11a-hexahydro (1,3) oxazolo (3,2-a) pyrido(1,2-d) pyrazine-8-carboxamide. It is an integrase inhibitor with dolutegravir-like carbamoyl pyridone structure. Cabotegravir is an antiretroviral medication used for the treatment of HIV/AIDS[3,4]. It is available as tablets and as intramuscular injection[5,6]. The U.S Food and Drug Administration authorized cabotegravir for per-exposure prophylaxis (PrEP) in at-risk individuals in December 2021 under the trade name Apretude. The structure of cabotegraviris shown in Figure 1.

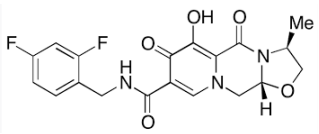

Fig. 1: Structure of cabotegravir

Rilpivirine is a non-nucleotide reverse transcriptase inhibitor[7] used especially for treating HIV- 1 infections in treatment-naive patients. It is a derivative of diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Chemically Rilpivirine is 4-[[4-[(E)-2- cyanoethenyl] - 2, 6-dimethyl-anilino] pyrimidin-

2-yl] amino] benzonitrile). The structure of Rilpivirine was shown in Figure 2.

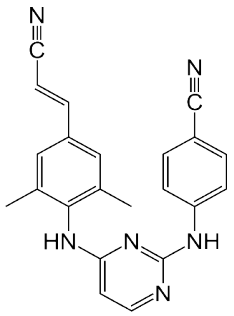

Fig. 2: Structure of Rilpivirine

For the treatment of human immunodeficiency virus type 1 (HIV-1) in adults, cabotegravir and rilpivirine are recommended[8]. The two medications are the first injectable long-acting antiretroviral medications. The combination injection is implied for maintenance treatment of HIV infection in adults who are receiving antiretroviral therapy and have undetectable blood HIV levels (viral load less than 50 copies/mL)[9], as long as the virus has not evolved a resistance to inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs).Review of the literature reveals that several LC-MS and FTIR assay techniques have been published for the quantification of cabotegravir and rilpivirine, both alone and in combination[10] with other medications. To the best of our knowledge, there is only one official method for estimating cabotegravir and rilpivirine[11,12] simultaneously by RP-HPLC in combined injection dosage form. Therefore, the authors made an attempt to develop a sensitive and cost-effective approach[13,14] for the simultaneous estimation and validation of cabotegravir and rilpivirine in an injection formulation in compliance with ICH recommendations.

EXPERIMENTAL

HPLC Instrument

HPLC Waters 2695 separation mode with T-60 UV-Visible spectrophotometer and a Rheodyne injector valve fitted with 10μL sample loop was used. Chromatographic separation was performed on Agilent C18 column (4.6 x150mm, 5μm). The column effluent was monitored with PDA detector set at 260nm and column temperature at 30°C

Chemicals and Reagents

All the chemicals and reagents in this experiment were of analytical grade. Water was double distilled and filtered with a membrane filter. Acetonitrile and potassium dihydrogen orthophosphate (Rankem) were used to prepare mobile phase. Pharmaceutical grade standard drugs

Preparation of 0.01N KH2PO4

The accurately weighed 1.42gm of potassium dihydrogen orthophosphate was transferred to a 1000ml volumetric flask and 900ml of mille-Q water was added and sonicated. Finally, volume was made up with water and pH was adjusted to 4.8 with a dil. orthophosphoric acid solution.

Preparation of mobile phase

Accurately measured 700ml (70%) of buffer solution, 300ml (30%) of HPLC grade acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45μm filter under vacuum filtration.

Diluents Preparation

Based on the solubility of the drugs acetonitrile and water in the ratio of 50:50 v/v was used as a diluent.

Preparation of standard solution

Accurately weighed 50mg of cabotegravir and 75mg of rilpivirine were transferred to 50 ml clean dry volumetric flask separately and 3/4th of diluents was added to the flask and sonicated for 10min. Volume was made up to the mark with diluent and labelled as standard stock solution (1000μg/mL of cabotegravir and 1500μg/mL of rilpivirine)

Preparation of working standard solutions

1mL from each stock solution was pipetted out and taken into a 10mL volumetric flask and volume was made up to the mark with diluent (100μg/mL of cabotegravir and 150 μg/mL of rilpivirine).

Preparation of Sample stock Solution

The sample solutions were prepared from injections vials of the drugs Cabotegravir (CAB) 600mg in 3ml inj vial & Rilpivirine (RILP) 900mg in 3mL vial. From injection vials 1ml of Rilpivirine

and Cabotegravir samples were transferred to a 100 volumetric flask, and 50ml of diluent was added to the flask and sonicated for 25 min, further the volume was made up with diluent and filtered using HPLC filter of 0.45μm pore size.

Preparation of Sample working solutions

0.5 mL from the above stock solution was pipetted out and was transferred into a 10 mL volumetric flask and made up to the mark using diluent to give 100μg/mL of cabotegravir and 150μg/mL of rilpivirine respectively.

Force Degradation Studies[15,16]

The degradation studies of cabotegravir & rilpivirine were carried out by following the guidelines of ICH. In order to determine the stability of cabotegravir & rilpivirine, the drug was treated with acid, alkali, hydrogen peroxide, heat, water

and UV light. Then the percentages of degradation were calculated.

Oxidative Degradation

1 ml of 20% hydrogen peroxide (H_2O_2) was added separately to 1 ml of rilpivirine and cabotegravir stock solution. The solutions were kept at 600 degrees Celsius for 30 minutes. The resultant solution was diluted to obtain 150 μg/mL and 100 μg/mL solutions for HPLC analysis, and 10 μl were injected into the system and chromatograms were recorded. The percentage degradation was then calculated to determine the sample's stability.

Acid Degradation Studies

Acid- and base-induced degradation was attempted by separately adding 1 ml of 2N Hydrochloric acid to the 1 ml of stock solutions of rilpivirine and cabotegravir, and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 150μg/mL & 100μg/mL solution and 10 μL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution rilpivirine and cabotegravir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 150μg/mL & 100μg/mL solution and 10 μl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 150μg/ml & 100μg/ml solution and 10μl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 1500μg/ml & 1000μg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 150μg/ml & 100μg/ml solutions and 10 μl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1h at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 150μg/ml & 100μg/ml solution and 10 μl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS & DISCUSSION

Method Development

The method was developed with different buffers and organic solvents but the composition of potassium dihydrogen ortho phosphate and acetonitrile showed symmetrical peaks, with good resolution-high theoretical plates, and low retention times of both rilpivirine and cabotegravir. The optimized parameters were shown in Table 1.

Table 1: Optimized Parameters

S.No	Parameters	Description / valve
1	Stationary phase	BDS (4.6*150mm,5μ)
2	Mobile phase	0.01NKH_2PO_4:Acetonitrile
3	Flow rate	1ml/min
4	Detection wave length	260nm
5	Detector	Photo diode array
6	Injection	Auto sampler
7	Injection volume	10μl
8	Column temperature	30∘C
9	run time	6 min

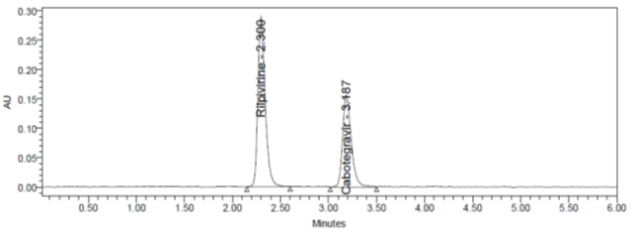

Fig. 3: Chromatogram of cabotegravir and rilpivirine sample solution

Method Validation[17,18]

The proposed RP-HPLC method was validated as per ICH guidelines.

System Suitability Constraints

The system suitability parameters were determined by preparing standard solutions of cabotegravir (100μg/mL) and Rilpivirine (150μg/mL) and the solutions were injected six times and the parameters like peak tailing, resolution and plate count were determined. The results were depicted in Table 2.

Specificity

Specificity of the method was determined by injecting blank to check whether peaks in the blank are eluting with drugs peaks and the chromatogram was shown in Figure 4.

Table 2: System Suitability Parameters of cabotegravir and rilpivirine

S.no	Rilpivirine			Cabotegravir			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.286	4310	1.30	3.150	5985	1.24	5.6
2	2.286	4366	1.28	3.173	6096	1.27	5.8
3	2.287	4461	1.31	3.173	6168	1.25	5.6
4	2.290	4534	1.31	3.175	6044	1.26	5.8
5	2.296	4546	1.27	3.179	6130	1.26	5.9
6	2.300	4348	1.28	3.187	6134	1.30	5.8

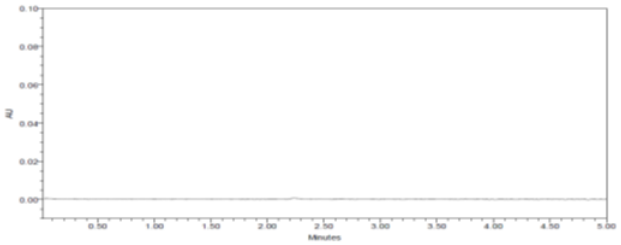

Fig. 4: Chromatogram of blank

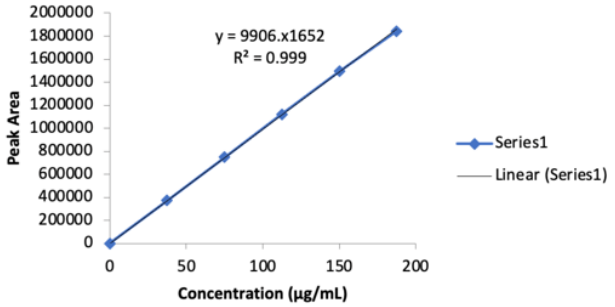

Fig. 5: Linearity plot for rilpivirine

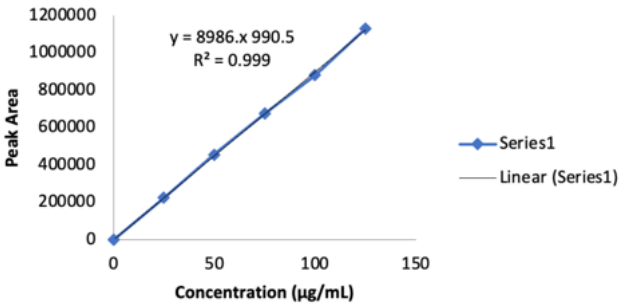

Fig. 6: Linearity plot for cabotegravir

Linearity

The linearity of cabotegravir and rilpivirine was in the concentration range of 25-150 μg/mL and 37.5-225μg/mL respectively with correlation coefficient (r^2) of 0.9999 for both the drugs. The results are given in Table 3 and calibration curves are shown Figures 5 and 6.

Table 3: Linearity table for Cabotegravir and Rilpivirine

Rlipivirine		Cabotegravir	
Concentration (μg/mL)	Peak area	Concentration (μg/mL)	Peak area
37.5	373564	25	225906
75	749001	50	457118
112.5	1121309	75	676784
150	1494597	100	882643
187.5	1843660	125	1131160
225	2244976	150	1351721

Precision

The precision of an analytical procedure is usually expressed as relative standard deviation (coefficient of variation) of a series of measurements

System Precision

It was performed by injecting a standard solution of cabotegravir and rilpivirine at working concentration of 100% six times that is 100μg/mL of cabotegravir and 150 μg/mL of rilpivirine). The % RSD was calculated for peak area and R_t and results are given in Table 4.

Method Precision

Six working sample solutions with the same concentrations were produced after multiple sampling from a sample stock solution. Each injection was given from a different working sample solution, and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.6% respectively for rilpivirine and cabotegravir. Data of method precision was given in Table 4.

Accuracy

The degree to which test findings acquired using an analytical method that are near to the actual value defines the method's accuracy. It is done by measuring the amount of pure drug recovered at three different concentrations (50%, 100% and 150%) in triplicate. The results are given in Table 5.

Table 4: Precision Data of Cabotegravir and Rilpivirine

S. No.	System Precision		Method Precision	
	Area of Cabotegravir	Area of Rilpivirine	Area of Cabotegravir	Area of Rilpivirine
1	902317	1514068	896827	1516275
2	907643	1501591	901873	1502459
3	908054	1517827	909842	1518279
4	901007	1519041	909086	1502783
5	906873	1517507	907337	1514846
6	907358	1517703	900895	1500996
Mean	905542	1514623	904310	1509273
S.D	3058.1	6598.9	5218.7	7978.1
% RSD	0.3	0.4	0.6	0.5

Table 5: Accuracy data of Cabotegravir and Rilpivirine

S. No.	Concentration level	% Recovery		Mean % Recovery	
		CAB	RIP	CAB	RIP
1	50%	99.9	101.4	100.25%	99.79%
		99.7	99.87		
		99.5	99.13		
2	100%	101.6	99.67		
		100.8	99.46		
		100.4	99.31		
3	150%	100.6	100.63		
		100.1	99.17		
		99.7	99.51		

Table 7: Assay Data of Cabotegravir and Rilpivirine

S. No.	Brand Name	Labeled claim	% Assay
1	Cabenuva	Cabotegravir (200mg/ml)	99.76
		Rilpivirine (300mg/ml)	99.55

*Avg of six determinations

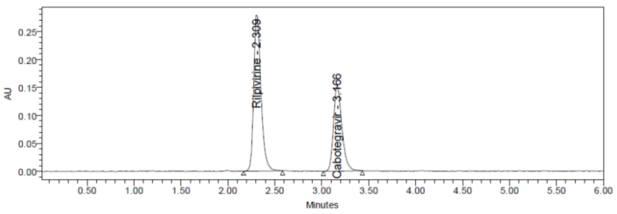

Fig. 7: Chromatogram of working sample solution

Robustness

Robustness of the proposed method has been evaluated by small deliberate changes in the system parameters such as flow rate, mobile phase composition, and temperature It was discovered that none of the aforementioned parameters changed the peak area, retention duration, or USP tailing even when the flow rate was changed by 0.1mL, the mobile phase was changed by 5%, or the temperature was changed by 5°C. The % RSD was found to be within the limits and the method was found to be robust. The robustness results were shown in Table 6.

Assay of marketed formulation of cabotegravir and rilpivirine

The validated RP-HPLC method was successfully applied for the assay of cabotegravir and rilpivirine in marketed formulations. The chromatogram for sample is shown in Figure 8. Assay results are represented in Table 7.

Forced degradation studies

According to ICH guidelines, the drug product underwent forced degradation in order to determine the method's ability to indicate stability, learn more about the degradation products, and demonstrate how the quality of a drug substance and drug product changes over time under the influence of various stressful conditions. According to ICH guidelines, the percentage of rilpivirine and cabotegravir that degraded was between 5% and 20%. The results of stress studies were shown in Table 8. The chromatograms of Forced Degradation studies were shown in Figure 8.

Table 8: Degradation data of Cabotegravir and Rilpivirine

Sample name	Rilpivirine Area	% degraded	Cabote-gravir area	% degraded
Standard	1514068	-	902317	-
Acid	692911	4.22	257464	4.10
Base	685421	5.26	248852	7.31
Peroxide	693933	4.08	253609	5.54
Thermal	711938	1.59	256497	4.46
Photo	712835	1.47	259588	3.31

Table 6: Results of Robustness

S. No.	Parameteres	Cabotegravir			Rilpivirine		
		RT	Plate coun t	TF	RT	Plate coun t	TF
1	Flow Rate 0.9	3.165	6156	1.26	2.283	4294	1.26
	Flow Rate 1	3.187	6134	1.3	2.3	4348	1.28
2	Temperature 27°C	3.17	6037	1.25	2.278	4540	1.26
	Temperature 30°C	3.187	6134	1.3	2.3	4348	1.28
3	Mobile Phase (-5%)	3.168	6181	1.27	2.286	4458	1.28
	Mobile Phase (+5%)	3.178	6111	1.24	2.288	4502	1.3

RT - Retension rime, TF - Tailing factor

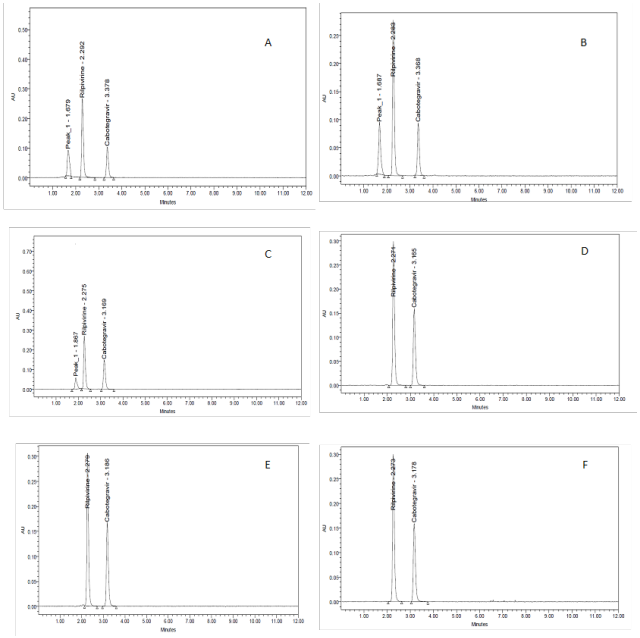

Fig. 8: Chromatograms of Forced Degradation studies, A) Acid degradation chromatogram, B) Base degradation chromatogram, C) Peroxide degradation chromatogram, D) Thermal degradation chromatogram, E) UV degradation chromatogram, F) Water degradation chromatogram

CONCLUSION

The simultaneous estimation of cabotegravir and rilpivirine in pharmaceutical dosage form was achieved using a simple, accurate, and precise method. Cabotegravir's retention time was determined to be 2.30 minutes, while retention time of Rilpivirine's was found to be 3.187 minutes, with %RSD of 0.3 and 0.4 respectively. For cabotegravir and rilpivirine recovery rates was 100.25% and 99.79% respectively. The technique created was straightforward and affordable, and it could be used for standard quality control tests in industries

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